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INVITED REVIEW

The Role of Molybdenum in Agricultural Plant Production

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• *Background* The importance of molybdenum for plant growth is disproportionate with respect to the absolute amounts required by most plants. Apart from Cu, Mo is the least abundant essential micronutrient found in most plant tissues and is often set as the base from which all other nutrients are compared and measured. Molybdenum is utilized by selected enzymes to carry out redox reactions. Enzymes that require molybdenum for activity include nitrate reductase, xanthine dehydrogenase, aldehyde oxidase and sulfite oxidase.
• *Scope* Loss of Mo-dependent enzyme activity (directly or indirectly through low internal molybdenum levels) impacts upon plant development, in particular, those processes involving nitrogen metabolism and the synthesis of the phytohormones abscisic acid and indole-3 butyric acid. Currently, there is little information on how plants access molybdate from the soil solution and redistribute it within the plant. In this review, the role of molybdenum in plants is discussed, focusing on its current constraints in some agricultural situations and where increased molybdenum nutrition may aid in agricultural plant development and yields.
• *Conclusions* Molybdenum deficiencies are considered rare in most agricultural cropping areas; however, the phenotype is often misdiagnosed and attributed to other downstream effects associated with its role in various enzymatic redox reactions. Molybdenum fertilization through foliar sprays can effectively supplement internal molybdenum deficiencies and rescue the activity of molybdoenzymes. The current understanding on how plants access molybdate from the soil solution or later redistribute it once in the plant is still unclear; however, plants have similar physiological molybdenum transport phenotypes to those found in prokaryotic systems. Thus, careful analysis of existing prokaryotic molybdate transport mechanisms, as well as a re-examination of know anion transport mechanisms present in plants, will help to resolve how this important trace element is accumulated.

Key words: Molybdenum, molybdate transport, nitrate reductase, Moco, *Vitis vinifera*, Merlot, Millerandage, sulfate transport, nitrogen fixation, nitrogen metabolism, plant nutrition.

INTRODUCTION

Molybdenum is a trace element found in the soil and is required for growth of most biological organisms including plants and animals. Molybdenum is a transition element, which can exist in several oxidation states ranging from zero to VI, where VI is the most common form found in most agricultural soils. Similar to most metals required for plant growth, molybdenum has been utilized by specific plant enzymes to participate in reduction and oxidative reactions. Molybdenum itself is not biologically active but is rather predominantly found to be an integral part of an organic pterin complex called the molybdenum co-factor (Moco). Moco binds to molybdenum-requiring enzymes (molybdoenzymes) found in most biological systems including plants, animals and prokaryotes (Williams and Frausto da Silva, 2002). The availability of molybdenum for plant growth is strongly dependent on the soil pH, concentration of adsorbing oxides (e.g. Fe oxides), extent of water drainage, and organic compounds found in the soil colloids. In alkaline soils, molybdenum becomes more soluble and is accessible to plants mainly in its anion form as MoO_4^-. In contrast, in acidic soils (pH <5·5) molybdenum availability decreases as anion adsorption to soil oxides increase (Reddy *et al*., 1997). When plants are grown under molybdenum deficiency, a number of varied phenotypes develop that hinder plant growth. Most of these phenotypes are associated with reduced activity of molybdoenzymes. These enzymes include the primary nitrogen assimilation enzymes such as nitrate reductase (NR), and the nitrogen-fixing enzyme nitrogenase found in bacteroids of legume nodules. Other molybdoenzymes have also been identified in plants including xanthine dehydrogenase/oxidase involved in purine catabolism and ureide biosynthesis in legumes, aldehyde oxidase (AO) that is involved in ABA biosynthesis, and sulfite oxidase that can convert sulfite to sulfate, an important step in the catabolism of sulfur-containing amino acids (Mendel and Haensch, 2002; Williams and Frausto da Silva, 2002). There are recent review articles on molybdoenzymes in plants, animals and prokaryotes (Mendel and Haensch, 2002; Williams and Frausto da Silva, 2002; Sauer and Frebort, 2003) that cover the extensive literature on the regulation and formation of Moco and the activity of Moco with molybdenum-dependent apoenzymes. Instead of re-examining this important component of molybdenum nutrition, this review will instead re-examine the effects of molybdenum nutrition in agricultural plants and explore the poorly understood aspect of molybdenum transport into and within the plant. In prokaryotes and lower-order eukaryotes, the molybdate transport systems have been well defined and are characterized at both the physiological, biochemical and genetic levels (Grunden and Shanmugam, 1997; Self *et al*., 2001). Unfortunately, this wealth of sequence information has not

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translated into an improved understanding of how eukaryotic systems transport molybdenum. This is not surprising as the primary molybdate transport systems present in prokaryotes are members of the ATP-binding cassette (ABC) protein superfamily. Members of this superfamily extend into plants; however, the numbers are large, where in arabidopsis alone there is predicted to be at least 129 putative proteins in the genome (Sanchez-Fernandez *et al*., 2001). Secondly a large number of other putative transport proteins that may encode molybdate transport systems still remain uncharacterized in sequenced plant genomes (Schwacke *et al*., 2003). Nevertheless, the prokaryotic systems are good starting points to discuss the types of eukaryotic systems that may exist and direct future research into specifically identifying plant molybdenum transport systems.

AVAILABILITY OF MOLYBDENUM IN AGRICULTURAL SOILS

Molybdenum is present in the lithosphere at average levels up to $2{\cdot}3\ mg\ kg^{-1}$ but can increase in concentration ($300\ mg\ kg^{-1}$) in shales that contain significant organic matter (Fortescue, 1992; Reddy *et al*., 1997). In agricultural soils, molybdenum is present as many different complexes depending on the chemical speciation of the soil zone. Mineral forms of molybdenum found in rocks include molybdenite (MoS_2), wulfenite ($PbMoO_4$) and ferrimolybdenite [$Fe_2(MoO_4)$] (Reddy *et al*., 1997). Release of molybdenum from solid mineral forms is through weathering, a process involving continual solution and oxidation reactions (Lindsay, 1979; Gupta, 1997*a*). Dissolved molybdenum available to plants is commonly found in the soluble MoO_4^- anion form (Lindsay, 1979). Above pH 4·23, MoO_4^- is the common anion followed in decreasing order by $MoO_4^- > HMO_4^- > H_2MO_4^0 > MoO_2(OH)^+ > MoO_2^{2+}$ (Lindsay, 1979). Once in solution, the MoO_4^- anion is subject to normal anion adsorption/desorption reactions, which are dependent on the specific chemistry of the soil solution. MoO_4^- can adsorb onto positively charged metal oxides (Fe, Al, Mn), clay minerals, dissolved organic compounds and carbonates. The adsorption of molybdenum onto positively charged metal oxides is strongly pH dependent with maximum adsorption occurring between pH 4 and 5 (K. S. Smith *et al*., 1997*b*). As the soil solution becomes more alkaline MoO_4^- availability increases. Every unit increase above pH 3, MoO_4^- solubility increases approx. 100-fold primarily through decreased adsorption of metal oxides (Lindsay, 1979). Consequently, the application of lime to agricultural soils has been an important tool to adjust soil pH and increase soluble molybdate.

Soluble MoO_4^- can also form ionic complexes with various ions in solution including Na, K, Ca and Mg, and can also be complexed with organic matter, particularly humic and fulvic acids (Jenne, 1977). The formation of these complexes can decrease the amount of MoO_4^- bound by metal oxides, increasing the amount of available MoO_4^- in solution (Reddy *et al*., 1997). Soil moisture also influences MoO_4^- availability where poorly drained wet soils (e.g. peat marshes, swampy organic rich soils) tend to accumulate MoO_4^- to high levels (Kubota *et al*., 1963). Many plants that grow under these soil conditions display high internal molybdenum levels, which can result in molybdenosis in ruminant animals if the material is used as animal feed (Scott, 1972; Gupta, 1997*a*). In contrast, well-drained sandy soils have been shown to leach significant amounts of applied molybdenum (Jones and Belling, 1967). The retention of molybdenum in sandy soils is very much pH dependent as acidic sands release neglible amounts of molybdenum in the leachate (Riley *et al*., 1987). Thus, soils rich in organic matter and with poor drainage traditionally accumulate soluble molybdate, while sandy soils are subject to molybdenum leaching but in a pH-dependent manner (Bloomfield and Kelso, 1973; Karmian and Cox, 1978; Riley *et al*., 1987).

IDENTIFICATION OF MOLYBDENUM AS AN ESSENTIAL PLANT ELEMENT

The requirement of molybdenum for plant growth was first demonstrated by Arnon and Stout (1939) using hydroponically grown tomato. Plants grown in nutrient solution without molybdenum developed characteristic phenotypes including mottling lesions on the leaves, and altered leaf morphology where the lamellae became involuted, a phenotype commonly referred to as 'whiptail' (Arnon and Stout, 1939). The only trace element that could eliminate these phenotypes was found to be molybdenum. The first reported case of molybdenum deficiency in an agricultural context occurred in mixed pasture grasses in the Lofty ranges of South Australia (Anderson, 1942). Local pastoralists reported significant failures of well-irrigated pastures containing subterranean clover (*Trifolium subterraneaum*), perennial rye grass and *Phalaris tuberosa*. These pastures had been sown on sandy loam (ironstone) soils, which were low in nitrogen, slightly acidic (pH 5·5–6), rich in iron oxides and had received significant superphosphate treatments in previous years (Anderson, 1942, 1946). It was noted at the time that clover could grow in these soils after liming or when wood-ash was present (Anderson, 1942). It was later identified that molybdenum was the most abundant trace element present in the soluble and insoluble extractions of the wood-ash. Molybdate application at 2 lb per acre was capable of increasing lucerne yields approx. 3-fold over control plots (Anderson, 1942). Shortly thereafter, Davies (1945) and Mitchell (1945) demonstrated that the whiptail phenotype in cauliflower could be overcome with the addition of molybdenum to the soil. Walker (1948) observed that tomato grown in molybdenum-deficient serpentine soils could be rapidly rescued (return of green colour, loss of mottling) with application of sodium molybdate directly to the soil, or by leaf painting and leaf infiltration.

In contrast, molybdenum toxicity in plants under most agricultural conditions is rare. In tomato and cauliflower, plants grown on high concentrations of molybdenum will have leaves that accumulate anthocyanins and turn purple, whereas, in legumes, leaves have been shown to turn yellow (Bergmann, 1992; Gupta, 1997*b*). The greatest concern associated with high plant molybdenum levels is with crops used for grazing or silage production. Ruminant animals, which consume plant tissues high in molybdenum content,

can suffer from molybdenosis, a disorder that induces copper deficiencies (Scott, 1972). Fortunately this disorder can be controlled by directly maintaining adequate Mo/Cu ratios in the rumen diet or by altering the availability of molybdenum to plants by changes in soil availability (pH adjustment).

VISUAL SYMPTOMS OF MOLYBDENUM DEFICIENCY IN PLANTS

Molybdenum deficiencies have been documented in many plant species where phenotypes range in severity and appearance (Hewitt and Bolle-Jones, 1952*a*). In the Brassicaceae family, molybdenum deficiencies are strikingly pronounced and reproducible amongst many of its members. Visual effects in young plants include mottling, leaf cupping, grey tinting, and flaccid leaves which are often found on seedlings that remain dwarfed until dying (Hewitt and Bolle-Jones, 1952*a*). In older plants, where deficiencies have been rescued or when deficiency levels are modest, the symptoms appear in younger leaf tissues with the characteristic loss of proper lamina development (whip-tail), leathery leaves and meristem necrosis (Hewitt and Bolle-Jones, 1952*b*). Investigation into the ultrastructure of leaves exhibiting whip-tail indicated that chloroplasts near the lesions became bulbous and enlarged with spherical protrusions bounded by chloroplast and tonoplast membranes (Fido *et al*., 1977).

Deficiency symptoms can also be masked by the indirect effect of molybdenum on nitrogen assimilatory enzymes (i.e. NR). Many horticultural, cereal and legume crops growing at deficient molybdenum levels in the presence of nitrate fertilizers will develop pale green leaves and, at times, necrotic regions at leaf margins with accompanied decreases in overall plant growth (Hewitt and Bolle-Jones, 1952*a*; Agarwala *et al*., 1978; Chatterjee *et al*., 1985; Chatterjee and Nautiyal, 2001). Molybdenum-deficient oat and wheat develop necrotic regions on leaf blades, and seeds are poorly developed and shrivelled (Anderson, 1956; Chatterjee and Nautiyal, 2001). In maize, molybdenum deficiency shortens internodes, decreases leaf areas and causes the development of chlorotic leaves (Agarwala *et al*., 1978). In reproductive tissues in maize, molybdenum deficiency can alter the phenotypes in developing flowers, including delayed emergence of tassels, small anthers, poorly developed stamens, and reduced pollen grain development (Agarwala *et al*., 1979). Pollen that is released from the anthers has been shown to be shrivelled and have poor germination rates (Agarwala *et al*., 1978, 1979). In grapevines, molybdenum deficiency has recently been suggested as the primary cause of a bunch development disorder called Millerandage or 'hen and chicken' (Williams *et al*., 2004). Millerandage (Fig. 1) is characterized by grapevine bunches that develop unevenly, where fully matured berries are present in a bunch alongside a large number of fertilized underdeveloped berries as well as unfertilized swollen green ovaries (Mullins *et al*., 2000). Millerandage has been reported primarily in *Vitis vinifera* 'Merlot' but unpublished anecdotal reports have suggested

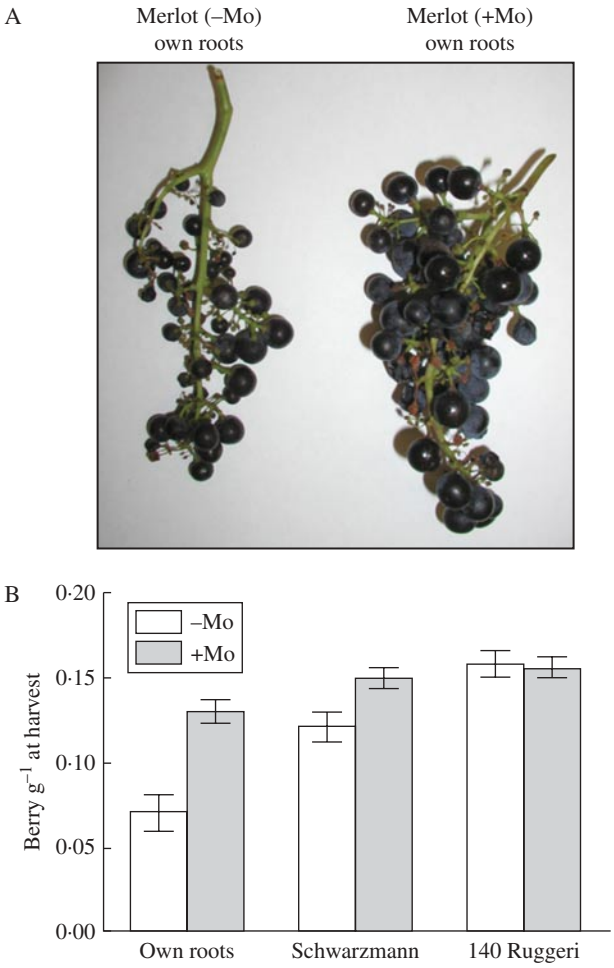

FIG. 1. Incidence of Millerandage in *V. vinifera* 'Merlot' and recovery from after foliar molybdate treatment and/or grafting onto rootstocks. Millerandage is identified by altered bunch development where berries within bunches at final harvest are at different developmental stages including fertilized matured ripened berries, fertilized but poorly developed berries and unfertilized enlarged green ovaries. (A) Merlot bunches at harvest displaying Millerandage in the (−Mo) treatment versus normal bunches in the (+Mo) treatment. (B) Final berry yields in response to foliar molybdenum treatments pre-flowering. Merlot vines were grown on own roots or grafted onto the rootstocks Schwarzmann and 140 Ruggeri.

the problem also occurs in Cabernet Sauvignon and Chardonnay cultivars (P. Dry, The University of Adelaide, Adelaide Australia, pers. comm.). In Merlot vines displaying Millerandage, other characteristic molybdenum-deficiency responses also appear including shortened zigzag-shaped internodes, pale-green leaves, increased cupped and flaccid leaves, and marginal leaf necrosis (K. Gridley, University of Adelaide, unpubl. res.).

BIOCHEMICAL RESPONSE IN PLANTS TO MOLYBDENUM DEFICIENCIES

Molybdenum deficiency affects plant metabolism at many different levels. The responses are strongly linked to the requirement of molybdenum for the various types of molybdoenzymes present in plants. Plant molybdoenzymes can be

broken down to those involved in nitrogen reduction and assimilation [i.e. nitrate reduction (nitrate reductase; NR), nitrogen fixation (nitrogenase), purine catabolism (xanthine dehydrogenase/oxidase; XDH), abscisic acid (ABA) and indole-3 acetic acid (IAA) synthesis (aldehyde oxidase; AO)] and sulfur metabolism (sulfite oxidase; SO). The molybdoenzymes can be classified even further based on their interactions with Moco. NR and SO contain a dioxo-Mo co-factor, which activates the protein when it is inserted into the protein complex (Mendel and Haensch, 2002). XDH and AO have a monoxo-Mo co-factor which requires Moco insertion and then subsequent sulfuration of the Mo centre to activate the Moco/protein complex (Mendel and Haensch, 2002). Since molybdenum is involved in a number of different enzymatic processes, a defined plant response to molybdenum deficiency can be complex and thus difficult to assign causally to specific enzyme systems. This is particularly evident in molybdoenzymes involved in nitrogen metabolism where overall reductions in plant growth and health can alter plant development, susceptibility to pest damage, and fruit or grain development (Graham and Stangoulis, 2005).

Molybdenum deficiency and NR activity

Molybdenum deficiencies are primarily associated with poor nitrogen health particularly when nitrate is the predominant nitrogen form available for plant growth. Inability to synthesize Moco will reduce the activity of the critical nitrogen-reducing and assimilatory enzymes including NR and XDH (Agarwala and Hewitt, 1954; Spencer and Wood, 1954; Afridi and Hewitt, 1964, 1965; Randall, 1969; Jones *et al*., 1976; Agarwala *et al*., 1978). In most plant species, the loss of NR activity is associated with increased tissue nitrate concentrations and a decrease in plant growth and yields (Spencer and Wood, 1954; Agarwala *et al*., 1978; Chatterjee *et al*., 1985; Unkles *et al*., 2004). Accordingly, in spinach plants grown under molybdenum-deficiency conditions, leaf NR activity was found to be reduced and overall final plant yields lower than control plants grown on adequate levels of molybdenum (Witt and Jungk, 1977). In wheat, molybdenum starvation was also shown to reduce maximum NR activities (lower potential V_{MAX}) irrespective of the regulatory control of NR by light and dark periods (Yaneva *et al*., 2000). Re-supplying molybdenum as a foliar spray or in supplemented nutrient solution in most instances will readily recover NR activity (Spencer and Wood, 1954; Afridi and Hewitt, 1964; Jones *et al*., 1976; Witt and Jungk, 1977). In the wine grapevine *Vitis vinifera* 'Merlot', poor growth during establishment and variable yields in mature plants grown in many South Australian vineyards is positively correlated with reduced petiolar molybdenum levels (Williams *et al*., 2004). Preliminary experiments by Ngaire Brady and colleagues (unpubl. res.) have demonstrated NR activity is significantly depressed in both Merlot shoots and roots even when grown with nutrient solution containing nitrate-N and adequate amounts of sodium molybdate (Fig. 2). It is believed that this is not the result of a mutation in the NR apoenzyme or in Moco biosynthesis as Merlot is capable of nitrate reduction when molybdenum is applied as a foliar treatment. Painting molybdate directly onto a leaf will induce NR activity in the treated leaf and in untreated leaves elsewhere in the canopy (Fig. 3). From this preliminary study, it would indicate the phenotype present in Merlot is not related to the synthesis and activity of Moco (Mendel and Haensch, 2002) or the NR apoenzyme but most likely associated with a disruption in the mechanism controlling molybdenum uptake and or internal redistribution in the xylem and or phloem. Interestingly, NR activity can also be rescued and plant growth returned to a 'normal' state by grafting Merlot onto hybrid North American rootstocks (Fig. 1). From this phenotype it would suggest the mutation in Merlot rests with its inability to readily accumulate molybdate from the soil solution.

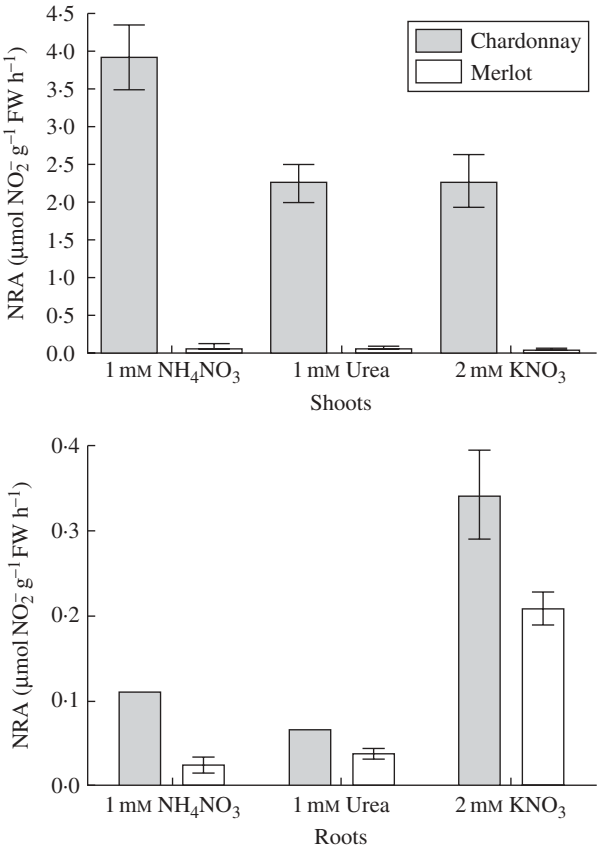

FIG. 2. *In vitro* nitrate reductase activity in grapevine leaves and roots. *In vivo* nitrate reductase activity (NRA) was measured in leaf discs and young root sections from Merlot and Chardonnay grapevines. The grapevines were grown in sand in pots and supplied modified Hoagland nutrient solution containing different nitrogen formulations. Data represents the mean ± s.e. ($n = 5$).

Molybdenum and its regulation of symbiotic nitrogen fixation

The other notable influence of molybdenum on plant nitrogen metabolism is in nitrogen-fixing legumes. The symbiotic bacterial enzyme nitrogenase is comprised of two subunits one of which is the MoFe protein directly involved in the reduction of N_2 to NH_3. Supply of molybdenum and

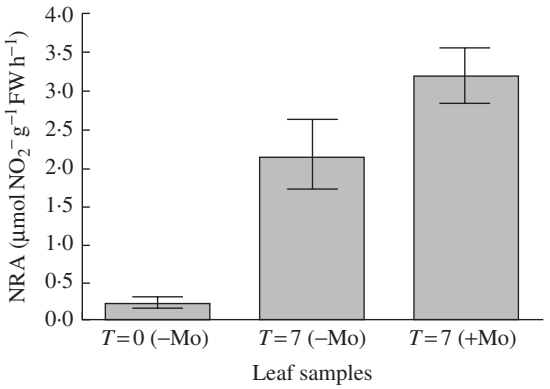

FIG. 3. Induction of nitrate reductase activity in Merlot leaves by foliar application of molybdate. The level of NRA in leaves of Merlot before and after application of molybdate to a single leaf. The leaves removed at $T = 0$ had no Mo applied. The leaves at $T = 7$ d either had molybdate (1·54 mM sodium molybdate) directly applied to them (+Mo) or were from the opposite side of the vine with no applied molybdate (−Mo). Data represents mean ± standard error ($n = 5$).

Fe to bacteroids is therefore an important process and most likely a key regulatory component in the maintenance of nitrogen fixation in legumes. Molybdate supplied by the plant must traverse nodule cellular membranes (plasma membrane and the peribacteroid membrane) as well as the bacteroid outer and inner membranes to reach the bacterial nitrogenase complex. A modABC transport system is most likely involved in bacteroid molybdate uptake; however, currently there is no information on the mechanism controlling molybdate transport into nodules and across the peribacteroid membrane. What is known, with respect to molybdenum and legume nitrogen fixation, is that molybdenum availability is closely correlated with nodule development (Anderson and Spencer, 1950; Anderson, 1956). In the absence of exogenous nitrogen (conditions which promote nitrogen fixation), molybdenum deficiency has been shown to significantly increase the number and size of clover nodules relative to control plants receiving molybdenum (Anderson and Spencer, 1950). Foliage of molybdenum-deficient clover also shows characteristic nitrogen-deficiency symptoms with pale green to yellow leaves and reduced biomass production (Anderson and Spencer, 1950; Hewitt and Bolle-Jones, 1952*a*). Legumes also appear to maintain molybdenum concentrations in nodules as the partitioning of molybdenum in common bean and soybean favours both nodules and developing seeds relative to other tissues (Gurley and Giddens, 1969; Franco and Munns, 1981; Ishizuka, 1982; Brodrick and Giller, 1991*b*). Foliar-applied molybdenum to common beans resulted in an 81 % increase in nodule molybdenum levels relative to the 56 % increase observed in the shoots (Brodrick and Giller, 1991*b*). It would thus appear that nodules are strong sinks for molybdenum, whether this is a direct consequence of an active nitrogenase enzyme is still to be determined. Experiments with soybean and common bean have shown that molybdenum fertilization can enhance the nitrogen-fixing symbiosis through increased nitrogenase activity rates and larger nodules (Parker and Harris, 1977; Adams, 1997; Vieira *et al*., 1998). However, subsequent increases in nitrogenase activity were not shown to occur as external molybdenum supply increased (Brodrick and Giller, 1991*b*). It would appear that nodules accumulate significantly more molybdenum than what is required in order to support bacterial nitrogenase activity and symbiotic nitrogen fixation.

The mobilization and export of fixed nitrogen out of the nodule requires the activity of the molybdoenzyme XDH. Depending on the legume species, fixed nitrogen is exported as either amides (glutamine and asparagine) or ureides (allantoin and allantoic acid), which are initially derived from the oxidative breakdown of purines. During this process, XDH catalyses the conversion of hypoxanthine to xanthine and xanthine to uric acid (Mendel and Haensch, 2002). The direct effects of molybdenum deficiencies on XDH activity in legume nodules is unknown; however, deficiencies would impact upon the ability of the plant to efficiently export reduced nitrogen from the nodule. XDH activity is also suggested to generate superoxide radicals (superoxide anions and/or hydrogen peroxide) in response to both biotic and abiotic stresses (Pastori and Rio, 1997; Hesberg *et al*., 2004). XDH activity has been shown to increase when phytopathogenic fungi infect both cereals and legumes. Whether this response is aimed at oxidative defence mechanisms it still unknown; however, in pea, XDH activity is strongly correlated with the activity of superoxide dismutase (Pastori and Rio, 1997). How this and other plant defence-related responses are linked to plant molybdenum nutrition is poorly understood. There is little direct evidence to conclude that improvements in plant molybdenum levels results in a decrease of disease, with the exception of small number of studies which indicate molybdenum fertilization can improve resistance to verticillium wilt in tomato (for a review, see Graham and Stangoulis; 2005). However, as discussed by Graham and Stangoulis (2005), this response may just be through improved plant health and not a direct effect on molybdenum in the defence response.

Molybdoenzymes not associated with nitrogen metabolism

Molybdoenzymes are also involved in the synthesis of the phytohormones ABA and indole-3-acetic acid (IAA). The Moco-dependent AO, catalyses the final steps in the conversion of indole-3-acetaldehyde to IAA, and the oxidation of abscisic aldehyde to ABA. Mutations in either the AO apoprotein or enzymes involved in Moco biosynthesis and Moco activation (sulfuration) will disrupt ABA synthesis (Marin and Marion-Poll, 1997; Schwartz *et al*., 1997; Sagi *et al*., 2002; Hesberg *et al*., 2004). Low ABA levels result in plants with a wilty appearance through excessive transpiration and loss of stomatal control, altered seed dormancy, and impaired defence responses (Mendel and Haensch, 2002). It has been shown recently the ABA-deficient mutants *flacca* and *aba3*, which both show wilty phenotypes, are disrupted in the Moco sulfuration step, which is required to activate the inserted Moco in AO (Bittner *et al*., 2001; Sagi *et al*., 2002). One of the distinct phenotypes in molybdenum-deficient Merlot is flaccid and cupped leaves similar to that observed in *flacca* and *aba3* (Robinson and Burne,

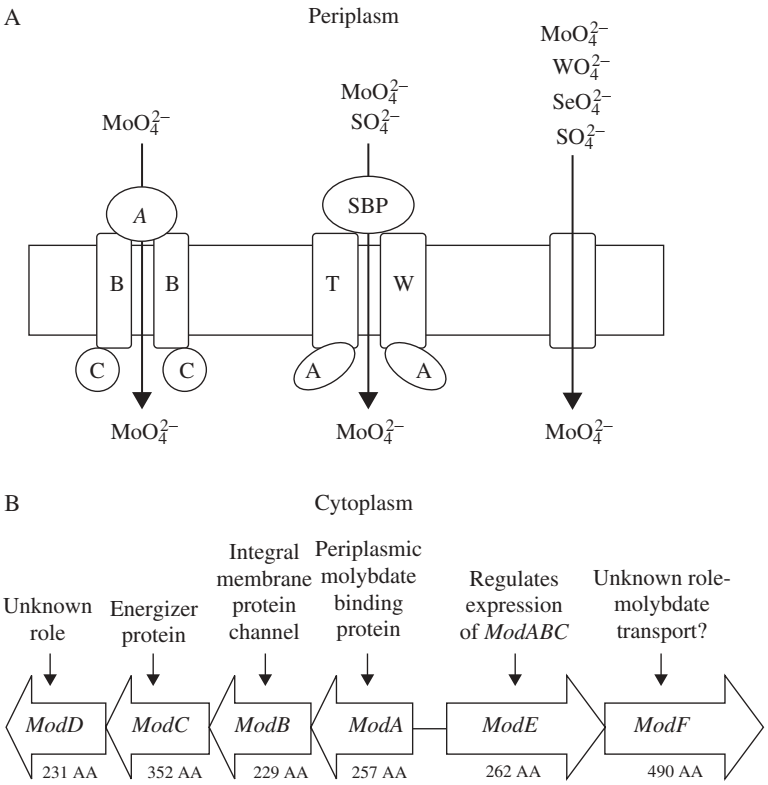

FIG. 4. Molybdate transport systems in *E. coli*. (A) Molybdate transport in *E. coli* is considered to involve three systems. The modABC protein complex, a sulfate transport complex similar to CYS UWA and an unidentified nonspecific anion channel. (B) Diagram of the *mod* operon present in *E. coli*.

2000). More research is required to ascertain whether AO activity in Merlot is affected by molybdenum deficiencies and the wilty phenotype associated with AO activity and sufficient ABA production.

MOLYBDENUM TRANSPORT

The mechanism(s) controlling molybdenum transport in plants and all higher-order organisms are still unknown. To date, molybdenum transport systems have only been identified and characterized in prokaryotes (bacteria) and some lower order eukaryotes (Self *et al*., 2001; Mendel and Haensch, 2002). In bacteria, the molybdenum transport system consists of multiple transport systems that ensure effective transfer of molybdenum into the cell. From studies in *Escherichia coli*, three systems are known to exist (Fig. 4), a primary high-affinity ABC-type transport system (ModABC) (Maupin-Furlow *et al*., 1995) and two secondary systems including an ABC-type sulfate transporter and a non-specific anion transporter (Maupin-Furlow *et al*., 1995; Self *et al*., 2001). Each of these proteins is encoded from genes found on a single operon (Maupin-Furlow *et al*., 1995; Walkenhorst *et al*., 1995). Downstream of the *ModABC* operon are two individual operons containing the regulatory genes *ModE* and *ModF* (Grunden *et al*., 1996). In many other bacteria and Archaea, *Mod* operons with similar or altered composition to that of *E. coli* have been identified through genome sequence homology (Grunden and Shanmugam, 1997; Self *et al*., 2001). However, only a few have been genetically and functionally characterized including *Mod* genes present in *Azotbacter vinelandii*, *Staphylococcus carnosus* and *Rodobacter capsulatus* (Luque *et al*., 1993; Wang *et al*., 1993; Neubauer *et al*., 1999).

ModABC consists of three proteins including a periplasmic molybdate-binding protein (ModA), an integral membrane channel protein (ModB) and an energizing protein (ModC). Molybdate binds to ModA (K_D *E. coli* approx. 20 μM) inducing a conformational change in the protein structure (Imperial *et al*., 1998). In *E. coli*, ModA will also bind tungstate but has a low affinity for similar-sized anions including sulfate (Rech *et al*., 1996; Imperial *et al*., 1998). ModB is an integral membrane protein containing five transmembrane spanning regions and a characteristic ABC signature motif (Self *et al*., 2001). The third component ModC, contains two Walker motifs (A and B) and an ABC motif similar to those found on ABC-type ATPases (Self *et al*., 2001). ModC is believed to be involved in the energization of molybdate transport. The ModABC complex is assumed to function as a molybdate transport system through an interaction between the channel protein ModB and the initial interactions between ModA and ModC through the conserved sequence motifs present in ModB (Fig. 4). In *E. coli* and a small number of other prokaryotes, ModABC is regulated by the activity of ModE which is a DNA transcriptional activator that is significantly more active when bound to molybdate

(Grunden *et al*., 1999; Self *et al*., 2001). The bound ModE–Mo complex represses the *ModABC* operon by binding to the ModA operator DNA and turning off molybdate transport (Grunden *et al*., 1999). *ModE* requires molybdenum to initiate the necessary conformational changes to become active, while other anions including tungstate or sulfate cannot effectively replace molybdenum binding (Grunden *et al*., 1999). *ModF* encodes a protein with an ABC signature motif similar to those found in the ABC–ATPase, ModC; however, its function is currently unknown (Self *et al*., 2001).

In *E. coli*, the K_M for molybdate is approx. 50 nM at pH 7·0 (Corcuera *et al*., 1993). The rate of molybdate uptake is influenced by the presence of molybdenum in the external medium where low concentrations (10 nM) enhance uptake and higher concentrations (approx. 1 μM) eliminate transport (Corcuera *et al*., 1993). In *E. coli* mutants lacking modABC activity, sulfate transporters can transport molybdate albeit at a lower affinity (K_M approx. 100 μM). In double mutants lacking both the modABC and sulfate transport systems, low affinity selenite-sensitive anion transporters can allow uptake of molybdate; however, the K_M for this transport phenomenon is not known (Lee *et al*., 1990). As a bacteroid in soybean root nodules, varied strains of *Bradyrhizobium japonicum* display different affinities for molybdate ranging between 45 nM and 0·36 μM (Lennox and Maier, 1987). The nitrogen fixing *Anabaena variabilis* accumulates molybdate at very low external concentrations in molybdenum-starved cells with an estimated K_M for molybdate of 0·33 nM (Thiel *et al*., 2002). The *A. variabilis* molybdate transport system can transport tungstate but not vanadate of sulfate (Thiel *et al*., 2002). In an *A. variabilis modBC* mutant, molybdate uptake is not detectable; however, after successive generations in sulfate-depleted medium, molybdate uptake can be restored and then later eliminated with sulfate re-supply (Zahalak *et al*., 2004). It would appear a second molybdate system such as a sulfate transporter may also participate in molybdate uptake in *A. variabilis* (Zahalak *et al*., 2004).

Molybdate transport into plants

Since there is no known molecular mechanism controlling molybdate transport in plants, and higher organisms for that matter, we are left to speculate on the types of systems based on the information we have from prokaryote and whole-plant molybdenum nutrition studies. Unfortunately, linking prokaryotic molybdate transport systems to the processes, which occur in eukaryotes, is not direct as there is limited sequence homology to modABC, modE and ModF in either arabidopsis or rice genomes or any other large plant expressed sequence tagged collections or partially sequenced genomes. However, there are similarities in physiological responses to molybdenum between prokaryotic and eukaryotic systems, namely the close interaction with sulfate transport. Sulfate is a similar-sized anion to molybdate, and evidence from prokaryotic studies suggests that sulfate transport systems and selenate-sensitive anion channels are capable of molybdate transport (Self *et al*., 2001). Stout and Meagher (1948) first demonstrated that, in tomato, molybdate (^{99}Mo) uptake in simple single salt buffer was significantly enhanced in the presence of phosphate and inhibited with sulfate. In a more representative nutrient solution where both phosphate and sulfate were present, sulfate was still found to be an effective competitor to molybdate uptake (Stout *et al*., 1951). In contrast, ^{99}Mo uptake into tomato increased when phosphorus was withheld from the nutrient solution which could be quickly reversed with phosphorus re-supply (Heuwinkel *et al*., 1992). From this study, it would appear molybdate is bound and transported across the plasma membrane using a phosphorus transport system. However, firstly, the competition studies demonstrated that when phosphorus levels were adequate, low concentrations of molybdate failed to effectively compete with phosphorus and, secondly, accumulated molybdate did not quickly move from roots to shoots and was instead readily available for exchange with non-labelled molybdate (Heuwinkel *et al*., 1992). These data suggest the phosphorus transport system may effectively bind and accumulate molybdate but would appear to have limited impact on molybdate transport under good growing conditions where the soil has adequate amounts of available phosphorus. It is also interesting to note that sulfate accumulation was significantly repressed during the phosphorus starvation period (Heuwinkel *et al*., 1992), a result which strengthens the case for the involvement of sulfate transport systems in molybdate transport. Since the initial observation by Stout and Meagher (1948), sulfate has since been shown to be an effective regulator of molybdenum uptake in many plants under a wide range of growing conditions (see review by Macleod *et al*., 1997). The similar size of the two anions and the relative concentrations in the soil solution most likely contribute to the competition observed with sulfate. However, the effect of sulfate on molybdate uptake is not solely at the root/soil interface. Soybean plants showed decreased molybdenum levels in aerial parts of the plant as the sulfate supply increased (Sing and Kumar, 1979) even if molybdenum was applied as a foliar spray (Kannan and Ramani, 1978).

The influence of other ions on molybdate uptake is poorly understood. In excised rice roots, the uptake of molybdate (0·01 mM) was significantly enhanced in the presence of 0·1 mM $FeSO_4$ but not in FeEDDHA (Patel *et al*., 1988). Interestingly, in free-living cowpea *Rhizobium* grown in iron-deplete conditions, the addition of high concentrations of molybdenum (1 mM) results in a release of a siderophore which appears to bind molybdenum and influences its uptake into the cell (Kannan and Ramani, 1978). Molybdate is highly mobile once in the plant where foliar absorption and translocation occur quickly. Williams (2004) showed that foliar-applied molybdate was rapidly distributed throughout the plant, including translocation towards the stem and roots within 24 h. Work completed by Ngaire Brady and colleagues (unpubl. res.) showed that foliar application of molybdate onto *V. vinifera* 'Merlot' restored NR activity in non-treated leaves elsewhere in the plant canopy (Fig. 3). Indeed, Brodrick and Giller (1991*a*), have shown good plant growth responses from foliar molybdenum application in the field. The mobility of molybdenum in plant tissues does appear to be genetically

controlled. Brodrick and Giller (1991*a*) observed different molybdate partitioning patterns between two *Phaseolus vulgaris* cultivars. One variety had a distinct advantage in distributing molybdate to developing seeds, nodules, roots and pod walls (Smith *et al*., 1995).

PUTATIVE PLANT MOLYBDATE TRANSPORTERS

The close interaction between molybdate and sulfate transport in many biological systems suggests a similar transport system is likely be involved in the movement of molybdenum into and within plants. The first plant sulfate transporters (*SHST1*, *SHST2*, *SHST3*) were identified from sulfur-starved roots of the tropical forage legume *Stylosanthes hamata* (Smith *et al*., 1995). The *SHST(1–3)* clones were identified by their ability to functionally complement a yeast sulfate transport mutant *YSD1* (Takahashi *et al*., 1996, 1999, 2000; F. W. Smith *et al*., 1997; Bolchi *et al*., 1999; Vidmar *et al*., 1999; Hawkesford, 2003). Since then a number of sulfate transport systems has been genetically identified and characterized in plants including genes from arabidopsis, barley, maize, potato, soybean and wheat (Hawkesford, 2003). In arabidopsis, there are 12 identified sulfate transporters with significant sequence homology and two more which are more distantly related (Hawkesford, 2003). This rich gene collection in many plant species has enabled distinct groups to be identified based on their sequences, cellular localization and response to sulfate (Takahashi *et al*., 1999). Group I sulfate transporters are high-affinity systems (K_M 1·5–10 μM) primarily expressed in roots, and increase or decrease in expression in response to sulfur starvation or supply, respectively. Group II sulfate transporters are considered low affinity systems (0·1–1·2 mM) based on their functional properties when expressed in yeast cells. Group II transporters also respond to sulfur starvation through increased expression levels. Group III transporters are mainly expressed in leaf tissues and account for five of the 14 sulfate-like transporters identified in arabidopsis. For the remaining two groups there is less information on their functionality in plants. Initial reports indicated a member of group IV (*AtSultr4;1*) may be targeted to chloroplasts (Shibagaki *et al*., 2002), while group V members are distantly related to members of group I–IV and no functional experimentation has been completed on them. The role of the sulfate transporter family in plants is slowly becoming clearer. Recently, the arabidopsis *AtSultr1;2*, which is a member of the group I sulfate transporters, was shown to be involved in sulfate uptake *in planta* where a T-DNA lesion in the *AtSultr1;2* locus allowed plants to grow on toxic concentrations of selenate and reduced its ability to accumulate sulphate into root tissues. There is an obvious requirement for more research into identifying the *in planta* function of the remaining sulfate transporters in plants before any of them can be nominated as putative molybdate permeases. However, one avenue of research that could be explored further is the role of these transport proteins when expressed in heterologous expression systems such as yeast cells. Although significant headway has been made in identifying genes encoding sulfate transport proteins very little information exists on the functional properties of most of these transporters in relation to anion selectivity, pH regulation and kinetic activities. Early studies in yeast demonstrated selenate and chromate as effective inhibitors of sulfate uptake (Breton and Surdin-Kerjan, 1977). Thus, selenate has been an effective screening tool to identify mutants that have disruptions in sulfate transport (Smith *et al*., 1995; Cherest *et al*., 1997). Using a selenate-resistant mutant *YSD1*, the selectivity of this mutant for sulfate transport and other anions such as molybdate is being explored. By removing molybdate from the media by activated charcoal scrubbing it has been possible to demonstrate that molybdate uptake at low external concentrations is also impaired in the yeast mutant (K. Gridley, unpubl. res.). This low molybdate media screen has been incorporated into ongoing experiments where selected plant sulfate transporters are being expressed in yeast and ranked on their ability to rescue growth on reduced molybdenum concentrations.

CONCLUDING REMARKS

Molybdenum nutrition is an essential component to healthy plant growth. Molybdate which is the predominant form available to plants is required at very low levels where it is known to participate in various redox reactions in plants as part of the pterin complex Moco. Moco is particularly involved in enzymes, which participate directly or indirectly with nitrogen metabolism. However, Moco is also uniquely involved in ABA synthesis where it has a significant effect on ABA levels in plant cells and consequently a role in water relations and transpiration rates through stomatal control and in stress related responses. There is significant scope in exploring practices, which optimize molybdenum fertilization in crops where nitrate is the predominant available N source or in nitrogen fixing legumes. There is also a large gap in the understanding of how molybdate enters plant cells and is redistributed between tissues of the plant. For instance the mechanism controlling molybdenum transport to nitrogen fixing bacteroids may be a unique control mechanism by which the plant can regulate the symbiosis indirectly through molybdenum availability to support nitrogenase activity. From our recent work with the grapevine cv. Merlot, we are starting to appreciate the influence of molybdenum on plant development and better understand mechanisms, which may be responsible for molybdenum uptake from the soil. It is ironic that it took a new industry to be expanded in South Australia where molybdenum first made its mark as an essential plant element to again reinforce the importance of molybdenum in plant development. Much more research is required to ascertain the simple processes involved in how plants gain access to molybdenum and how the element may be used in the future to expand growing areas where soil molybdate profiles limit plant growth.

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